

IN THE CLAIMS:

Claims 1-10 (*Cancel*)

11. (*New*) A method of excluding uncleaved electrophoretic probes from an electrophoretic separation in an assay for detecting the presence or absence of one or more polynucleotides in a sample, the method comprising the steps of:

providing for each polynucleotide a primer specific for a first region of the polynucleotide and an electrophoretic probe specific for a second region of the polynucleotide, the electrophoretic probe having a capture ligand attached and a releasable eTag reporter attached, such that upon release the eTag reporter of each electrophoretic probe has an electrophoretic mobility different from that of the eTag reporters of every other electrophoretic probe so that eTag reporters from different electrophoretic probes form distinct peaks upon electrophoretic separation;

combining in a mixture a nuclease, the sample, the electrophoretic probes, and the primers under conditions that allow the primers and the electrophoretic probes to hybridize to their respective polynucleotides to form complexes, the nuclease recognizing such complexes and digesting the electrophoretic probes therein so that eTag reporters are released; and

adding to the mixture a capture agent that specifically binds the capture ligands of the electrophoretic probes and confers on the undigested electrophoretic probes a charge that causes the undigested electrophoretic probes to migrate upon electrophoretic separation in a direction opposite of that of the eTag reporters, thereby excluding said undigested electrophoretic probes the electrophoretic separation of the released eTag reporters.

12. (*New*) The method of claim 11 wherein said eTag reporter has a molecular weight of from 150 to 10,000 daltons.

13. (*New*) The method of claim 11 wherein said eTag reporter of each electrophoretic probe having a negative charge upon release therefrom and said capture agent confers on said undigested electrophoretic probes a positive charge.

14. (*New*) The method according to 13 wherein each of said electrophoretic probes is defined by the formula:

(D, M)-N-T

wherein:

(D, M)-N is said eTag reporter;

D is a detection group;

M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, phosphorus, nitrogen, sulfur, and boron;

N is a nucleotide; and

T is an oligonucleotide specific for said second region of said polynucleotide, each T having a length in the range of from 12 to 60 nucleotides.

15. (New) The method of claim 14 wherein said one or more polynucleotides is in the range of from 5 to 100 polynucleotides, and wherein said eTag reporter is defined by the formula:



wherein D is a fluorescent label, N is a nucleotide, and M is said mobility modifier.

16. (New) The method of claim 15 wherein said fluorescent label is a fluorescein.

17. (New) The method in accordance with claim 13, 14, or 15 wherein said capture ligand is biotin and said capture agent is avidin.

18. (New) The method in accordance with claim 13, 14, or 15 wherein said capture ligand is an antigen and said capture agent is an antibody or antibody fragment that binds specifically to the antigen.

19. (New) The method of claim 13 wherein each of said electrophoretic probes is defined by the formula:



wherein:

D-M-N is said eTag reporter;

D is a detection group;

M is a mobility modifier having a molecular weight in the range of from 30 to 3,000 daltons;

N is a nucleotide; and

T is an oligonucleotide specific for said second region of said polynucleotide, each T having a length in the range of from 12 to 60 nucleotides.

20. (New) The method of claim 19 wherein: (i) D is a fluorescent label, (ii) said capture ligand is biotin and said capture agent is avidin, (iii) M is a mobility modifier having a molecular weight in the range of from 35 to 1500 daltons, and (iv) said one or more polynucleotides is in the range of from 5 to 100 polynucleotides.